

Supplemental Information

Materials. Boc-amino acids were purchased from Peptides International and Bachem. 2-(1H-benzotriazolyl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU) was from American Bioanalytical. Boc-Cys(4-MeBzl)-OCH₂-PAM resin was prepared from the following starting materials: Boc-Cys(4-MeBzl)-OCH₂-φ-CH₂COOH (NeoMPS, Inc.) and polystyrene aminomethyl resin (100-200 mesh, 1% DVB, Rapp Polymere GmbH). Reduced and oxidized glutathione and N,N-diisopropylethylamine (DIEA) were from Fluka. Ultra pure urea was purchased from ICN Biochemicals, cyanogen bromide (CNBr) from Sigma Aldrich. Alexa Fluor 350 carboxylic acid succinimidyl ester was obtained from Invitrogen. Solvents used for peptide synthesis and purification, namely, dimethylformamide (DMF, sequencing grade), dichloromethane (synthesis grade) and acetonitrile (HPLC grade) were all from Fisher Scientific. Lyophilized *S. aureus* ATCC 29213 was acquired from two different sources: Becton Dickinson and Microbiologics. Recombinant anthrax lethal factor was purchased from List Biological Laboratories, Inc. A sequence-optimized chromogenic substrate of lethal factor, Ac-NleKKKVLP-pNA, was synthesized as previously described. HIV_{BaL} gp120, expressed in T-RExTM-293 cells and affinity purified, was a generous gift from Profectus Biosciences, Inc.

Table S1. Crystallization and cryo-protection conditions.

Mutant	Reservoir solution	Cryo-protectant solution
Y3A	0.1 M sodium cacodylate trihydrate pH 6.5; 1.4 M sodium acetate trihydrate	30% glycerol; 0.1 M Na cacodylate trihydrate pH 6.5; 1.4 M sodium acetate trihydrate
I6A	2% PEG 400; 0.1 M HEPES-Na, pH 7.5; 2 M ammonium sulfate	15% glycerol; 2% PEG 400; 0.1 M HEPES-Na, pH 7.5; 2 M ammonium sulfate
Y16A	25% PEG 4,000; 0.2 M ammonium sulfate; 0.1 M sodium acetate, pH 4.6	20% glycerol; 25% PEG 4,000, 0.2 M ammonium sulfate; 0.1 M sodium acetate, pH 4.6
Y21A	30% PEG 8,000; 0.2 M ammonium sulfate	30% glycerol; 30% PEG 8,000; 0.2 M ammonium sulfate
Q22A	30% PEG 8,000; 0.1 M sodium cacodylate trihydrate pH 6.5; 0.2 M magnesium acetate tetrahydrate	20% glycerol; 30% PEG 8,000; 0.1 M sodium cacodylate trihydrate pH 6.5; 0.2 M magnesium acetate tetrahydrate
R24A	20% PEG 4,000; 0.1 M sodium citrate tribasic dehydrate; 20% iso-propanol	20% glycerol; 20% PEG 4,000; 0.1 M sodium citrate tribasic dehydrate; 20% iso-propanol
W26Abu	30%MPD; 0.1 M HEPES sodium pH 7.5; 0.2 M sodium citrate dihydrate	30%MPD; 0.1 M HEPES sodium pH 7.5; 0.2 M sodium citrate dihydrate
W26Ahp	0.1 M sodium cacodylate trihydrate pH 6.5; 0.2 M sodium citrate tribasic dehydrate; 30% isopropanol	30% glycerol; 0.1 M sodium cacodylate trihydrate pH 6.5; 0.2 M sodium citrate tribasic dehydrate; 30% isopropanol
F28A	25% PEG 4,000; 0.2 M ammonium sulfate; 0.1 M sodium acetate, pH 4.6	20% glycerol; 25% PEG 4,000, 0.2 M ammonium sulfate; 0.1 M sodium acetate, pH 4.6

Table S2. Crystallographic data and model refinement statistics

	Crystal							
PDB Code	I6A 3LVX	Y16A 3LO1	Y21A 3LO2	Q22A 3H6C	R24A 3LO4	W26Abu 3LO6	W26Ahp 3LO9	F28A 3LOE
Diffraction Statistics								
Space group	F432	C222	P2 ₁ 2 ₁ 2	I222				
Cell dimensions, Å								
a,	118.07	33.46,	43.68,	46.52,	46.63,	45.60,	46.15,	31.53,
b,	118.07	74.65,	29.88,	48.35,	48.67,	31.02,	30.65,	39.83,
c	118.07	25.87	41.55	24.70	24.56	39.73	39.82	46.26
Molecules/a.u.	2	1	2	2	2	2	2	1
Resolution ^a , Å	50-1.63 (1.69-1.63)	50-1.56 (1.59-1.56)	50-1.56 (1.59-1.56)	50-1.63 (1.66-1.63)	50-1.75 (1.78-1.75)	50-1.56 (1.62- 1.56)	50-1.56 (1.62- 1.56)	50-1.56 (1.59- 1.56)
Number of reflections								
Total	16,546	8,865	12,822	11,611	10,735	15,445	15,108	7,952
Unique	9,275	4,857	7,547	6,731	5,962	8,441	8,262	4,342
R _{merg} ^b , %	10 (79.0)	9.0 (19.2)	10.1 (10.6)	6.1 (22.1)	5.7 (56.8)	5.0 (9.6)	5.9 (13.2)	8.7 (9.8)
Completeness, %	99.6 (96.6)	99.3 (87.3)	92.9 (80.3)	90.8 (84.1)	99.3 (97.2)	99.4 (94.4)	97.0 (88.2)	99.2 (83.5)
Redundancy	10.9 (5.7)	13.3 (12.2)	3.1 (2.9)	3.8 (3.7)	5.8 (5.0)	6.7 (6.3)	6.3 (6.2)	6.7 (6.0)
I/σ, I	17.4 (2.0)	27.9 (16.4)	10.3 (10.6)	12.6 (6.7)	25.7 (2.8)	24.9 (25.0)	20.7 (18.8)	20.1 (18.6)
Refinement Statistics								
Resolution, Å	20-1.63	20-1.60	20-1.56	15-1.63	15-1.75	20-1.56	15-1.56	15-1.56
R ^c , %	16.5	18.3	17.4	17.8	17.1	16.9	18.9	17.4
R _{free} ^d , %	18.1	22.8	19.8	21.2	20.9	19.8	20.8	18.4
No of atoms								
Non-hydrogen atoms	591	278	563	557	540	567	528	270
Protein	470	231	462	476	464	476	466	232
Water	101	33	77	64	60	81	64	38
RMS deviations								
Bond lengths, Å	0.017	0.019	0.013	0.018	0.016	0.014	0.015	0.015
Bond angles, °	1.67	1.71	1.63	1.83	1.58	1.66	1.73	1.55
Mean B-value (overall) Å ³	11.93	20.78	13.43	17.48	22.99	13.78	12.56	14.92
Ramachandran plot								
Most favored region, %	95.8	91.7	95.8	100	95.8	93.8	95.2	100
Additional allowed	4.2	8.3	4.2	0	4.2	6.2	4.8	0

^aAll data (outer shell).

^bR_{merge} = $\sum |I - \langle I \rangle| / \sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections after rejections

^cR = $\sum |F_o| - |F_c| / \sum |F_o|$, where F_o and F_c are the observed and calculated structure factors, respectively

^dR_{free} = defined by Brünger

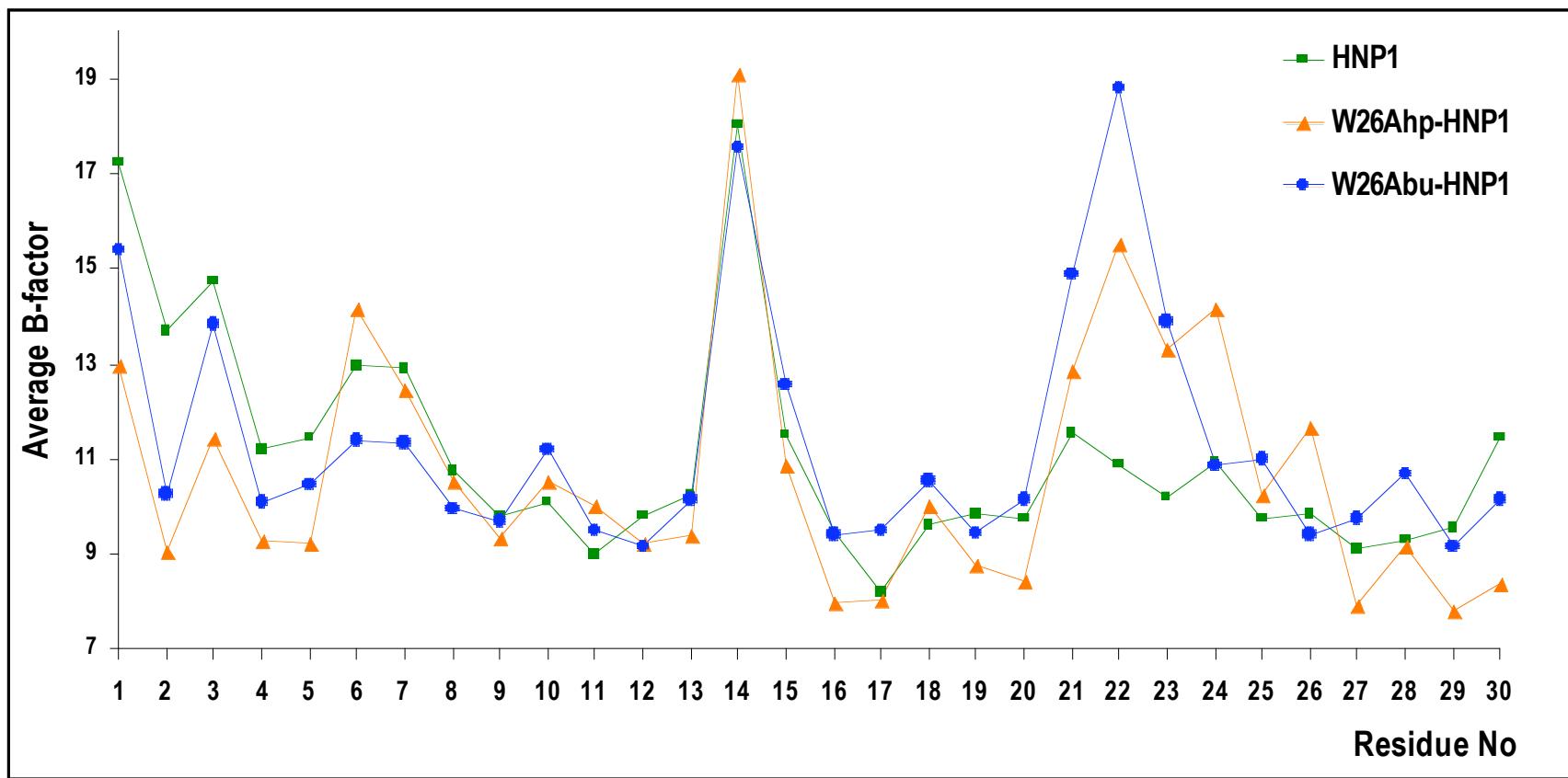


Figure S1. Averaged B-factor values of residues in wild type HNP1, W26Ahp-HNP1 and W26Abu-HNP1. Values refer to the B-factors of all atoms and are averaged over two crystallographically independent monomers present in the asymmetric unit of crystal.